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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No 0704-0188

1. AD-A208 883

1b. RESTRICTIVE MARKINGS

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3. DISTRIBUTION/AVAILABILITY OF REPORT
Distribution Unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
N/A

5. MONITORING ORGANIZATION REPORT NUMBER(S)
N/A

6a. NAME OF PERFORMING ORGANIZATION	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
University of British Columbia	N/A	Office of Naval Research

6c. ADDRESS (City, State, and ZIP Code)	7b. ADDRESS (City, State, and ZIP Code)
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8a. NAME OF FUNDING/SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
Office of Naval Research	ONR	N00014-87-J-0127

8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS			
	PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
800 N. Quincy St. Arlington, VA 22217-500	6153 N	RR94196	4412021	

11. TITLE (Include Security Classification)
Characterization of Biofouling Marine Caulobacters and their Adhesive Holdfast

12. PERSONAL AUTHOR(S)
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13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 3/1/87 TO 2/28/89	5/30/89	4

16. SUPPLEMENTARY NOTATION
N/A

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Biofouling; holdfast; Caulobacters, adhesives; biofilms (KT)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)
The goals of this project were a) To isolate marine Caulobacter bacteria and characterize them by physiological and genetic criteria, b) analyze the adhesive holdfast organelles of various isolates via isolation and chemical analysis and cloning of holdfast related genes, and c) develop and characterize the capabilities of marine Caulobacters for molecular genetic manipulation. This includes methods of introduction of genes and plasmids and the development of expression vectors. (Keywords:)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	U

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c OFFICE SYMBOL
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DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

89 6 12 123

Final Report on Contract N00014-87-G-0127
Thursday, May 25, 1989

Principle Investigator John Smit

Contractor University of British Columbia

Contact Title Characterization of Biofouling Marine Caulobacters and their Adhesive Holdfast

Start Date 1 March 1987

Research Objectives a) To isolate marine Caulobacter bacteria and characterize them, b) analyze the adhesive holdfast organelle, c) Develop and characterize the capabilities of marine Caulobacters for molecular genetic manipulation.

Progress

On the distribution and characteristics of Caulobacters in the marine environment

There is no reliable way to precisely quantitate either the absolute abundance of Caulobacters in an environment or relative fraction of the total marine bacterial population. This is owing to the fact that Caulobacters are difficult to identify in rapid screening methods, the fact that they are caught up in complex aggregations of bacteria that cannot be adequately dissociated for individual culturing and the fact that no more than 50% of the bacteria noted in the ocean can subsequently be grown by traditional bacteriological culturing methods. Nevertheless, we continued a qualitative effort to isolate Caulobacters from approximately 40 sites, most along the Western coast ranging from San Diego, California to Vancouver. We were always able to isolate Caulobacters from a seawater sample and conclude that they are ubiquitous in at least shallow nearshore regions of the marine environment and that most, if not all, can be cultured readily. We learned that approximately half can grow in anaerobic conditions (contrary to the established literature), about 1/3 have significant resistance to Hg^{2+} via inducible mercury reductases, only a few harbor native plasmids.

Analysis of the adhesive holdfast produced by Caulobacters

We completed a preliminary study of the composition of the holdfasts of marine and freshwater Caulobacters, using lectin binding, inhibition of lectin binding by characterized saccharides and by evaluating sensitivity to a variety of glycolytic and proteolytic enzymes. We concluded that all marine Caulobacters have N-acetylglucosamine in their holdfast and nothing else detectable by lectins (although surely there are other substituents). Moreover, the N-acetylglucosamine is present as stretches of 3 or more units. We have found no enzyme that has any effect on the holdfasts, including chitinases, which normally cleave polymers of N-acetyl glucosamine. This is perhaps understandable from an ecological standpoint, since chitinases are very commonly produced by other marine bacteria. It will be intriguing to discover the biochemical basis for this resistance to degradation. Freshwater Caulobacters showed more variability in holdfast composition. The holdfast of some strains bound lectins other than N-acetylglucosamine-specific ones. Even those that bound N-acetylglucosamine-specific lectins were distinguishable from marine Caulobacters in that the holdfasts were sensitive to chitinases and lysozymes. Apparently, the polymeric regions of N-acetylglucosamine are not modified to prevent enzymatic attack in these freshwater strains.

We have also been developing methods to isolate the polysaccharide in quantity for in-depth chemical and structural analysis. We concentrated on a procedure based on the observation that holdfasts bind very tightly to colloidal gold particles. Once bound, the holdfast is no longer adhesive and the complex can be isolated readily with CsCl density centrifugation, relying on the

high density imparted by the gold binding. However, the method did not work adequately well with wild type cells. We attempted to dissociate holdfast from the main cell body by extensive sonication. This proved to be inadequate; antisera raised against the holdfast-colloidal gold complexes produced sizable activities to the lipopolysaccharide coat of Caulobacters.

The solution to this problems seems to be a mutant we isolated after UV mutagenesis. This mutant line sheds its holdfast into the medium which can be collected by attachment to the colloidal gold particles.

On the adhesive properties of the holdfast. We also initiated studies aimed at discerning what types of surfaces to which the Caulobacters will attach. This was approached by the preparation of glass surfaces covalently modified with a variety of chemical substituents (provided by Dan Rittschoff, Duke University Marine Labs), resulting in surfaces ranging from highly charged to very hydrophobic. A quantitative static flow attachment assay was developed. Among the things learned was that Caulobacters will attach to virtually all surfaces at some frequency, but appear to prefer substrates that are moderately hydrophobic. Freshwater Caulobacters attach better to very hydrophobic surfaces than do marine Caulobacters. By growing marine strains that tolerate low ionic strength media in a freshwater medium, we learned that the salts in seawater are apparently responsible for lowered adhesiveness to hydrophobic surfaces. Of practical significance was the finding that dimethyldichlorosilane treated glass (ie classical "silanizing") was reasonably effective in discouraging attachment, a convenience for many future experiments, especially holdfast isolation procedures.

On the genes that specify the holdfast structure During the project we began to isolate holdfast-related genes from selected freshwater and marine strains. This required development of a rapid screening technique for detecting holdfast defective strains. Two methods were devised, one involves attaching colonies to cellulose acetate plastic and staining those bacteria that remain after a vigorous washing (in a search for those that fail to remain attached). The other procedure involves attachment of colonies to glass fiber filters and subsequent staining with Congo Red dye, which appears to be reasonably specific to the holdfast of marine Caulobacters.

We also initiated steps to prepare transposon-mutagenized populations of Caulobacters. The use of a transposon insertion allows rapid detection and isolation of the specific gene affected. We prepared a library of 16000 independent insertions in a freshwater Caulobacter strain, isolated 78 holdfast-defective mutants and began genetic analysis of the mutants to learn where the transposon was inserted.

On the development of molecular genetic capabilities of marine Caulobacters. Very little was known about the capabilities for genetic manipulation of this group of marine bacteria. Such information is needed, not only for the isolation and characterization of holdfast genes, but also to proceed with a number of other biotechnology-related ideas related to positive uses of biofilm forming bacteria. During the project we completed studies aimed at discerning which strains of marine Caulobacters were most suitable for conjugal transfer of plasmids (an essential method needed to introduce foreign genes), what antibiotic resistance markers can be expressed in these bacteria, and at what concentrations (necessary for cloning experiments) and what gene promoters can be recognized in selected strains (necessary for the expression of foreign genes). We also determined which transposons can be used to generate mutants in marine Caulobacters, ie, which will efficiently transpose to random genomic locations.

The general conclusions were encouraging; we found no significant difficulties in selecting suitable strains for most standard genetic manipulations, most antibiotics can be used, the promoter for a freshwater Caulobacter gene (a highly expressed surface protein) is recognized by

many of the marine Caulobacters and can be used for expression vector construction and transposons Tn5 and Tn7 at least transpose in several of our marine strains. Based on these and other observations, we have selected a single strain, MCS6, to be the focus for future development of molecular genetic capabilities in marine Caulobacters.

Publications (relevant to the ONR funding)

Abstracts

•Nivens, D.E. , A. Tunlid, M.J. Franklin, J. Smit, and D. White, 1988. Infrared monitoring of interactions between Caulobacter species and solid surfaces. Abstracts of the 88th Annual Meeting, American Society for Microbiology, 1988.

•Mitchell, D., C. Ong and J. Smit, 1988. Isolation and characterization of Caulobacter holdfast mutants. Abstracts of the annual meeting, Northwest Branch, American Society for Microbiology.

Publications

•Smit, J. 1987. "Caulobacters in the marine environment" *in* Marine Biodeterioration: Advanced Techniques Applicable to the Indian Ocean. Edited by the American Institute of Biological Sciences, Oxford and IBH Publishing, New Delhi, India.

•Anast, N. and J. Smit. 1988. Isolation, characterization of marine Caulobacters and assessing the potential for genetic experimentation. Appl. Environ. Micro. 54: 809-817.

•Merker, R. M. and J. Smit, 1988. Analysis of the adhesive holdfast of marine and freshwater Caulobacters. Appl. Environ. Micro. 54, 2078-2085.

•Fisher, J., J. Smit and N. Agabian, 1988. Transcriptional analysis of the major surface array gene of Caulobacter crescentus. J. Bacteriol. 170, 4706-4713.

Manuscripts currently in preparation

•Merker, R. M., D. Rittschoff and J. Smit. Analysis of the attachment of marine and freshwater Caulobacters to surfaces using chemically defined substrates. for Appl. Environ. Microbiol.

•Nivens, D.E. , A. Tunlid, M.J. Franklin, J. Smit, and D. White. Infrared monitoring of interactions between Caulobacter species and solid surfaces.

•Ong, C and J. Smit. The adhesive holdfast of Caulobacters: Isolation and analysis of mutants defective in the attachment of the organelle to the cell.

•Gilchrist, A., W. Bingle and J. Smit. Introduction of plasmids into freshwater and marine Caulobacters by electroporation.

Training Activities

Two graduate students and one postdoctoral were supported by this contract.

Awards

•Appointment of John Smit as an associate member of the Oceanography Department, University of British Columbia, July 1987.
•Appointment of John Smit to the Education Committee of the Canadian Society of Microbiologists, June 1988.

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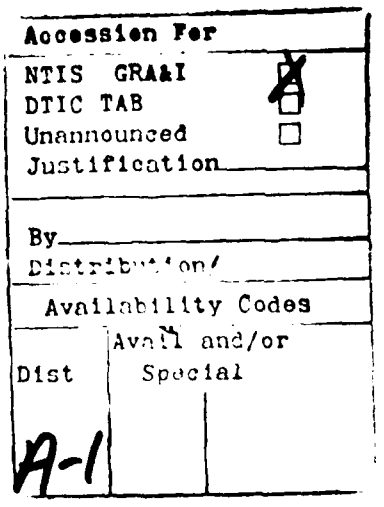

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